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Mitochondrial DNA sequences support species status for the Indian Spotted Eagle *Aquila hastata*

by Ülo Väli

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The taxonomic status of the Indian Spotted Eagle *Aquila* (*pomarina*) *hastata* has been an issue of dispute. Originally described as a species, *Morphnus hastatus* Lesson, 1834, it was subsequently considered a subspecies of Lesser Spotted Eagle *A. pomarina* C. L. Brehm, 1831. Nominate *A. p. pomarina* breeds mainly in eastern and central Europe, and in the Middle East, and is entirely migratory, whilst *A.* (*p.*) *hastata* is a sedentary form restricted to India. The breeding ranges of the two are separated by thousands of kilometres, preventing any study of the reproductive barrier between them, the most important difference according to the Biological Species Concept. Parry *et al.* (2002) found a number of morphological differences between the two taxa, which led them to propose specific status for *A.* (*p.*) *hastata*. The most striking difference noted was in gape width, smallest in *A. p. pomarina*,

TABLE 1
Sequences used in the current study.

Species	Locality	Accession no. in GenBank	Source
Aquila (*p.*) *hastata*	India	AY987286	Lerner & Mindell (2005)
A. p. pomarina	Germany	AJ604490	Seibold *et al.* (1996)
A. p. pomarina	Estonia	DQ462413	this study
A. clanga	Pakistan	AY987284	Lerner & Mindell (2005)
A. clanga	Estonia	DQ462414	this study
A. c. chrysaetos	Switzerland	AJ604486	Seibold *et al.* (1996)
Ictinaetus malayensis	unknown	AY754056	Bunce *et al.* (2005)
Lophaetus occipitalis	South Africa	AJ604502	Helbig *et al.* (2005a)

TABLE 2
Kimura 2-parameter nucleotide distances (above diagonal) with standard errors (below diagonal) between six eagle taxa according to cytochrome-*b* gene sequences.

	1	2	3	4	5	6
1. *A.* (*p.*) *hastata*		0.036	0.036	0.050	0.053	0.083
2. *A. p. pomarina*	0.006		0.018	0.038	0.050	0.076
3. *A. clanga*	0.006	0.004		0.040	0.051	0.078
4. *I. malayensis*	0.007	0.006	0.007		0.054	0.078
5. *L. occipitalis*	0.008	0.007	0.007	0.008		0.073
6. *A. c. chrysaetos*	0.010	0.009	0.010	0.010	0.009	

intermediate in Greater Spotted Eagle *A. clanga* Pallas, 1811, a closely related but separate species, and largest in *A.* (*p.*) *hastata*.

Genetic analyses

Molecular markers are, in most cases, supposed to reflect a true phylogenetic descent (Avise 1994). The divergence between mitochondrial DNA lineages of *A. clanga* and *A. p. pomarina* suggests these species split *c.*1 MYA (Seibold *et al.* 1996). However, no genetic data for *A.* (*p.*) *hastata* have been available for comparison. Recently, two extensive phylogenetic analyses of eagles have been published, by Helbig *et al.* (2005a) and Lerner & Mindell (2005). Unfortunately, these studies did not analyse jointly *A. p. pomarina* and *A.* (*p.*) *hastata*, and, hence, did not clarify the systematics of the three spotted eagle taxa, although the need for further studies was elucidated by these authors.

I analysed 995 bp of nucleotide sequence from the mitochondrial cytochrome-*b* gene of *A. clanga*, *A. p. pomarina* and *A.* (*p.*) *hastata*, and included also their closest monophyletic relatives, Indian Black Eagle *Ictinaetus malayensis* and Long-crested Eagle *Lophaetus occipitalis* (Helbig *et al.* 2005a, Lerner & Mindell 2005), as well

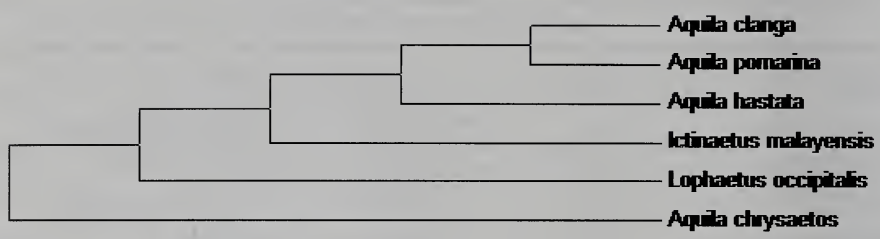

Figure 1. Phylogenetic maximum parsimony tree of six eagle species based on cytochrome-*b* sequences.

as a more distant member of the genus *Aquila*, *A. chrysaetos*, to the analysis. I used data available in GenBank and sequenced Estonian samples of *A. clanga* and *A. pomarina* to diversify the geographical range of sampled individuals' origin (Table 1). Phylogenetic analysis was made using MEGA version 3.1 (Kumar *et al.* 2004).

Of the 995 nucleotides, 49 were variable amongst the three spotted eagle taxa. According to pairwise Kimura 2-parameter distances, cytochrome-*b* genes of *A. clanga* and *A. p. pomarina* differ by 1.8% (Table 2, see also Seibold *et al.* 1996). *A.* (*p.*) *hastata* differs from both by 3.6%. Comparatively, single nucleotide substitution was found between studied *A. p. pomarina* samples (0.1% difference), and two within *A. clanga* (0.2%). All spotted eagles were clearly separated from the two *Lophaetus* group eagles, and from *A. chrysaetos*. Phylogenetic reconstructions clearly suggest that *A.* (*p.*) *hastata* separated from *A. clanga* and *A. p. pomarina* before their differentiation. The topology of the retrieved phylogenetic tree was the same using different approaches, and bootstrap analysis supported the earlier separation of *A. hastata* by 97% (minimum evolution), 96% (neighbour joining) or 92% (maximum parsimony; Fig. 1).

Taxonomy and conservation

No precise divergence limit to assign species or subspecies rank to avian taxa exists, but usually cytochrome-*b* sequences of subspecies differ by no more than 3% (Helbig 2000), which is less than the divergence between *A. p. pomarina* and *A.* (*p.*) *hastata*. Although phylogenetic trees of single genes do not always reflect species evolution and deeper genetic analyses, based on a wider range of markers and using more individuals, are needed in the future, the analysis of the mitochondrial cytochrome-*b* gene supports those morphological and ecological studies that have distinguished *hastata* specifically (Parry *et al.* 2002), as its difference to other spotted eagles is larger than between those two at this locus. Using the mean supposed evolutionary rate of avian mitochondrial coding DNA, 2% per one million years (Lovette 2004), the split of *A. hastata* could have happened *c.*1.8 MYA.

There is also the question as to whether *A. clanga* and *A. p. pomarina*, which are known to hybridise in the wild and produce fertile offspring (Väli & Lõhmus 2004, Helbig *et al.* 2005b), represent different species. However, in the area of sympatry, the reproductive barrier in taxon level between *A. clanga* and *A. pomarina* persists, and the possible mechanism could be the lower fertility of female hybrids or backcrosses (Helbig *et al.* 2005b), which probably prevents gene-flow even after a breakdown in ecological barriers. Therefore, it is recommended to continue to treat *A. clanga* and *A. pomarina* specifically. These two northern species arose during interglacial periods of the Pleistocene, after diverging from the southern members of the *Lophaetus* group (Helbig *et al.* 2005a), including *A. hastata*, thereby supporting the southerly origin (southern Asia, Africa) of *Lophaetus* eagles.

Obviously, the revised taxonomic status of *A. hastata* should be taken into account in planning further conservation action to protect this highly endangered bird with a world population of probably fewer than 100 pairs (Prakash 1996). The species should be treated separately from *A. pomarina*, which according to the IUCN Red List is of Least Concern (BirdLife International 2004).

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Mandatory changes to the scientific names of three Neotropical birds

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Application of the *International code of zoological nomenclature* (ICZN 1999) requires changing the specific names for Dusky-billed Parrotlet *Forpus sclateri,* Glittering-bellied Emerald *Chlorostilbon aureoventris* and Guianan Toucanet *Selenidera culik.* Art. 23.9 (Reversal of Precedence) clarifies the proper usage of nomenclature in each of the three cases.

Forpus sclateri

Peters (1937) used *Forpus sclateri* (Gray, 1859, type from the río Javari, Peru) as the oldest available name for Dusky-billed Parrotlet. He also introduced the name *F. s. eidos* as a *nomen novum* for *Psittacula modesta* (Cabanis, 1848, type from British Guiana), which he considered a secondary junior homonym of *Psittacula modesta* (Fraser, 1845). Fraser's name applies to the Long-tailed Parakeet *Psittacula longicauda modesta* of Enggano Island, Sumatra, and Cabanis' *modesta* to the genus *Forpus,* Boie, 1858. Thus, these authors independently applied the name *modesta* to different nominal genera (*Psittacula* Cuvier, 1800 [type: *Psittacus alexandri* Linnaeus] and *Psittacula* Illiger, 1811 [type: *Psittacus passerinus* Linnaeus]), obviating homonymy. Because Cabanis' name has been employed as valid since 1899 (e.g. Ihering & Ihering 1907, Snethlage 1914, Cory 1918, Naumburg 1930, Pinto 1938), ***Forpus modestus*** must be considered the valid name for Dusky-billed Parrotlet, with *sclateri* as a subspecies and *eidos* a synonym.

Chlorostilbon aureoventris

As noted by Steullet & Deautier (1946) and Mallet-Rodrigues (2005), Shaw (1812) described *Trochilus lucidus* based on material described informally (not under the